Proteomics Using Mass Spectroscopy
Can the FEL Help?

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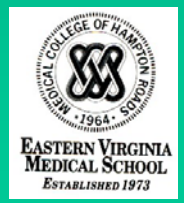
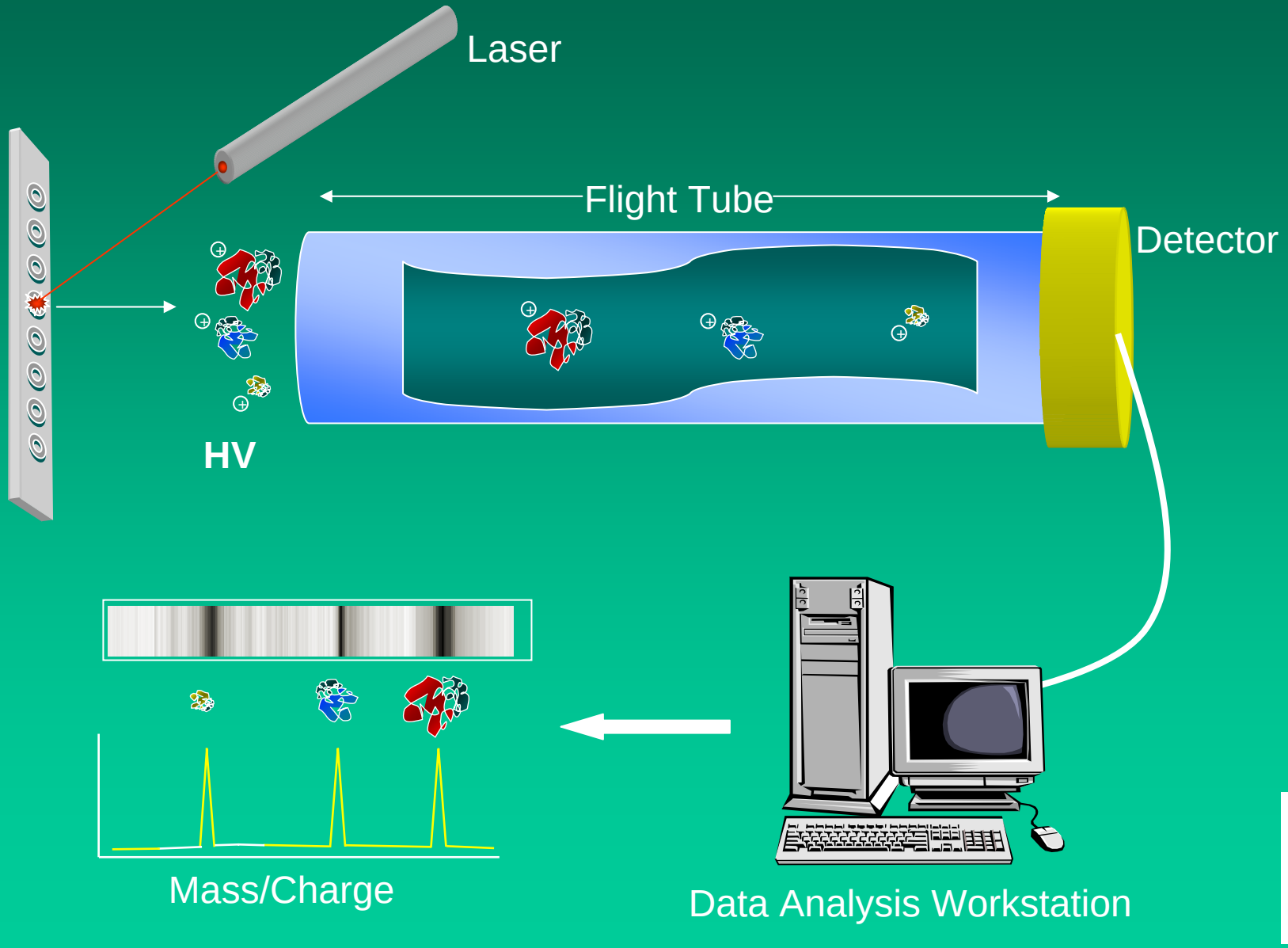


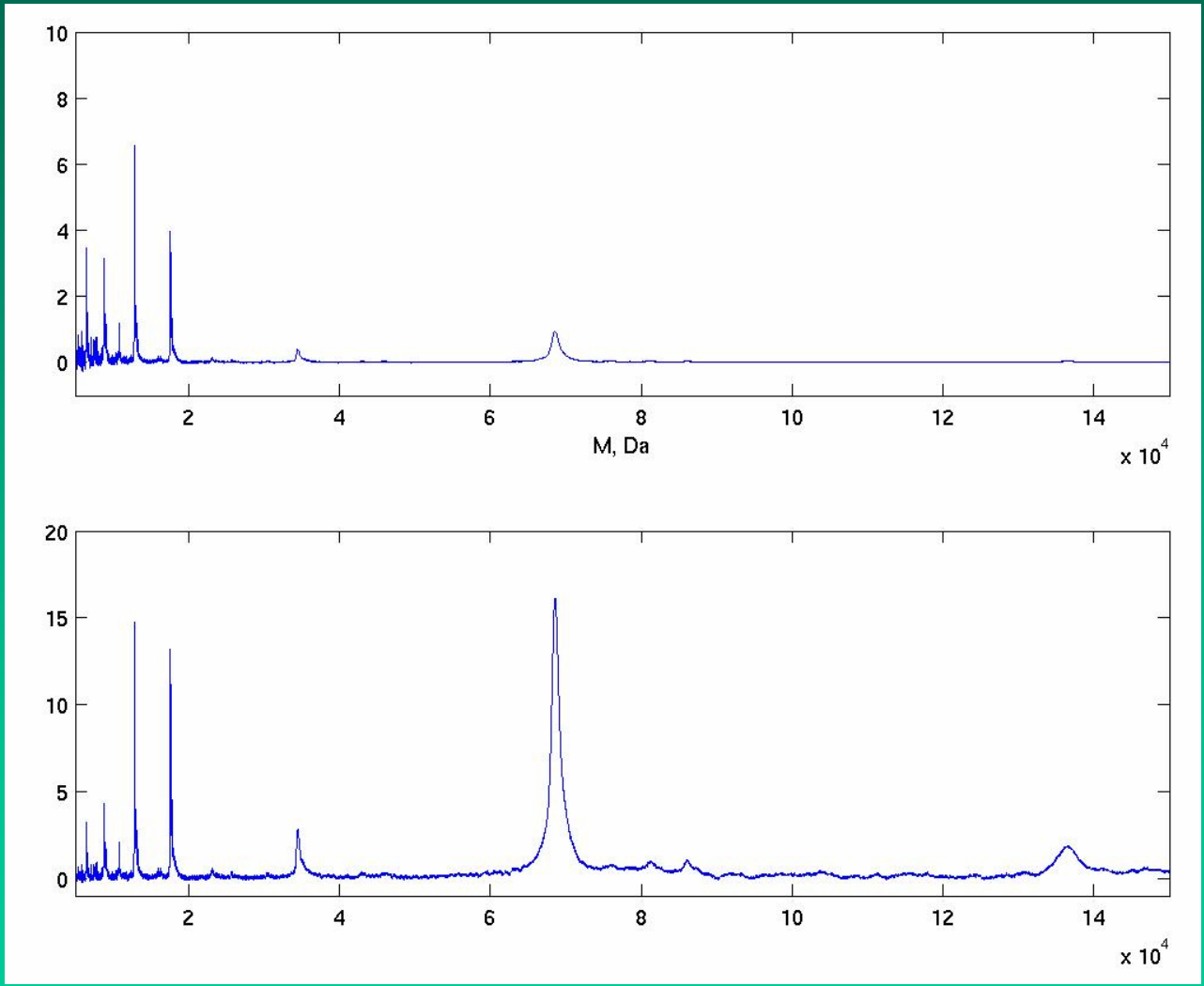
Surface Enhanced Laser Desorption/Ionization (SELDI) Time-Of-Flight (TOF) Mass Spectroscopy



W&M Mass Spectroscopy Proteomics Goals

- Enhance Signal/Noise
- Connect markers to biological processes

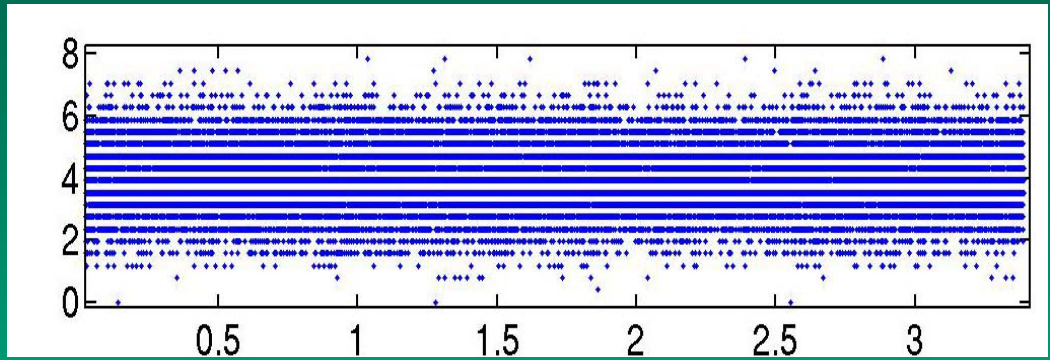
Data processing



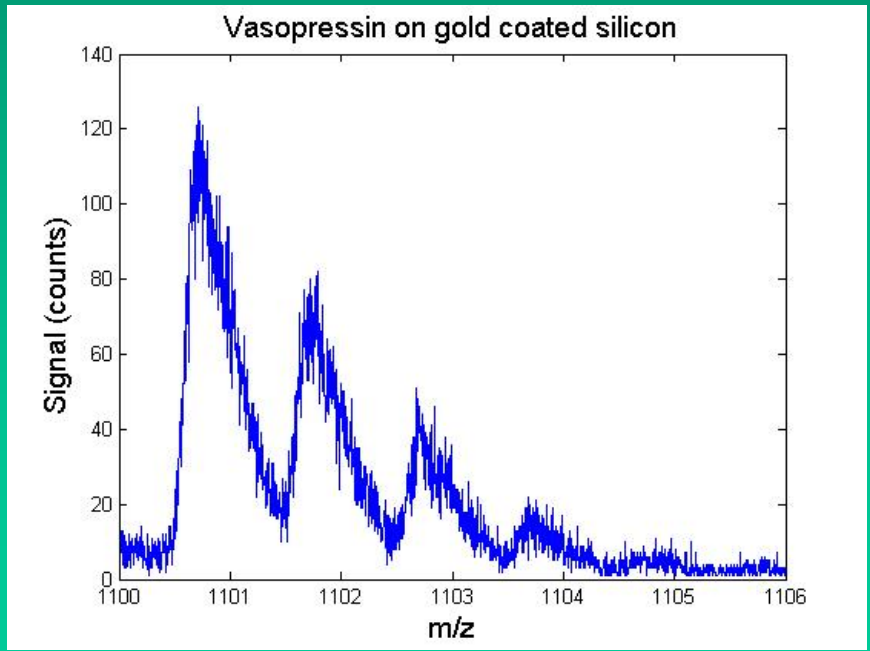
- Resampling improves peak identification

Can the FEL help?

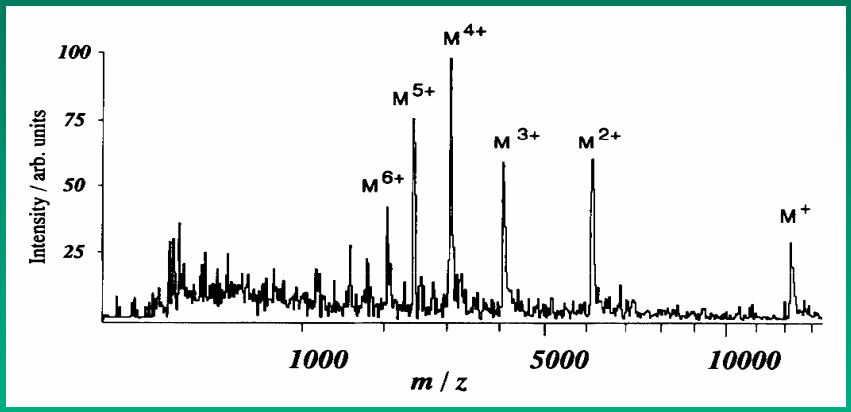
- Analog electronics and detectors



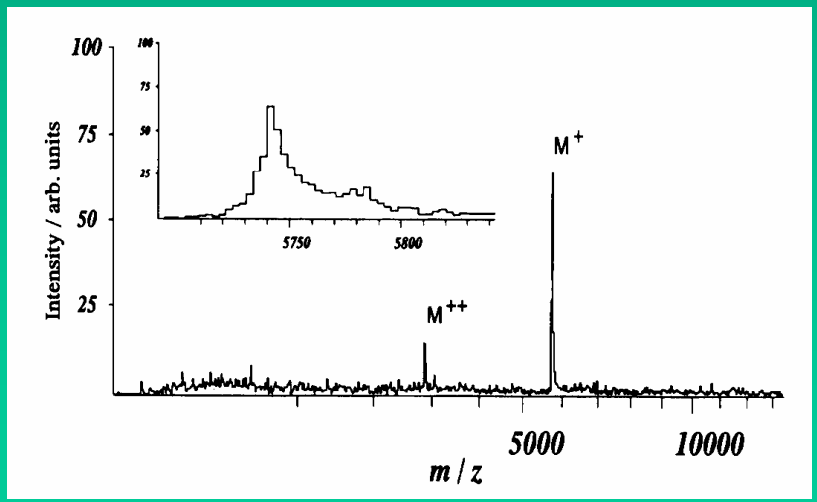
- High rep rate FEL could lead to ion counting



Haglund et al. use the Vanderbilt FEL for MALDI



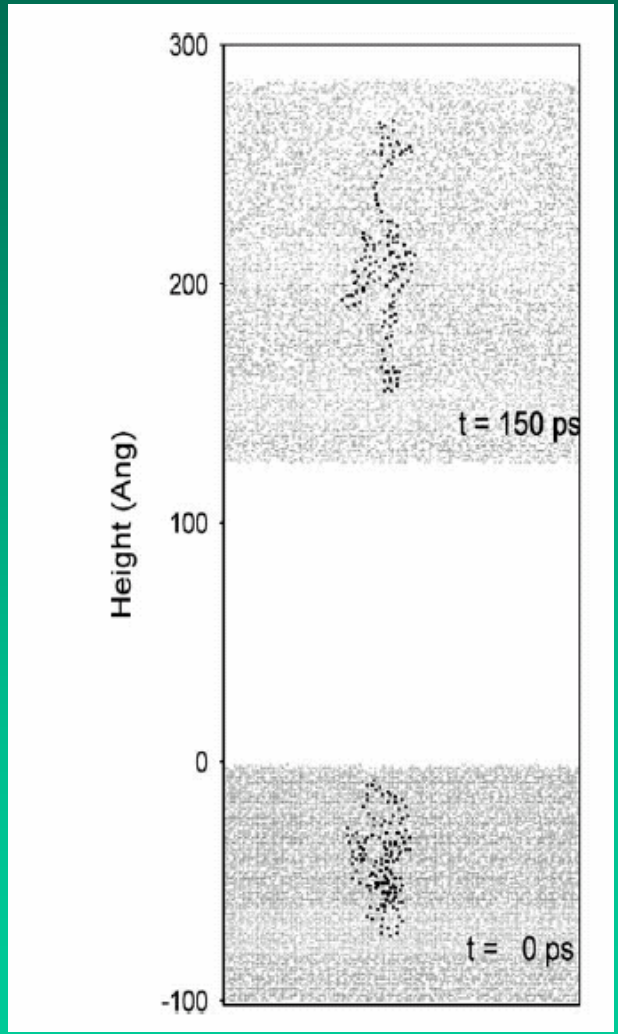
- TOP: IR-MALDI spectrum of cytochrome-C in succinic acid (single shot). Note the appearance in the spectrum of multiply-charged ions with high efficiency.



- BOTTOM: Ten-shot MALDI spectrum of insulin in succinic acid using a special fast-drying matrix preparation to make micron-size crystallites. Note the absence of matrix background. Mass resolution m/Dm~600.

Ionization is complicated!

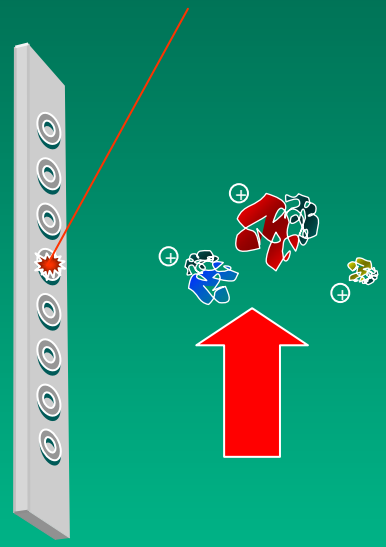
- Large pulse energies
- Collisional ionization
- Big spots needed!



B.J. Garrison, A. Delcorte, L.V. Zhigilei, T.E. Itina, K.D. Krantzman, Y.G. Yingling, C.M. McQuaw, E. J. Smiley, and N. Winograd, *Appl. Surf. Sci*. 203-204, (2003).

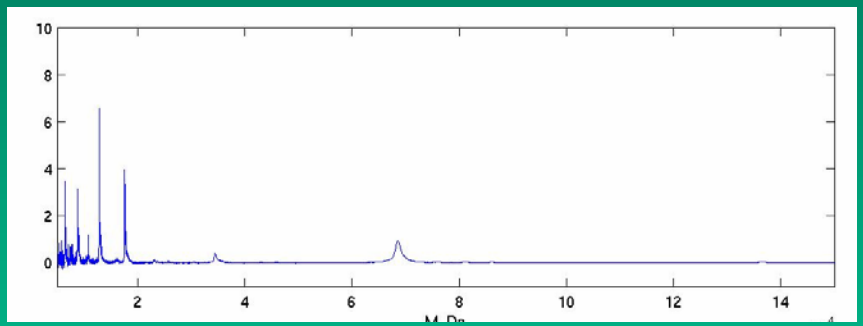
Can the FEL help?

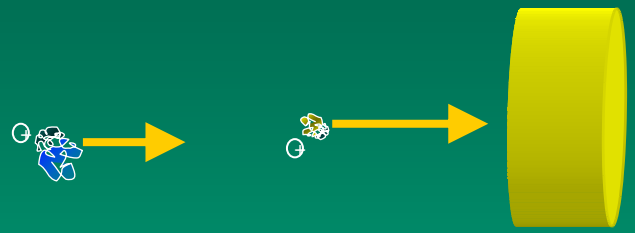
- Source photoionization to enhance the signal
- High rep rate and high power could make single ion counting possible (everything ionizes at a 10^{14} W/cm$^{2)}$
- What is the role of wavelength?



Limits to SNR

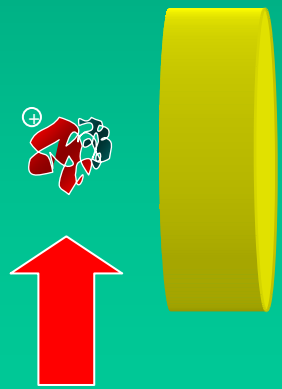
- Detector gain variation



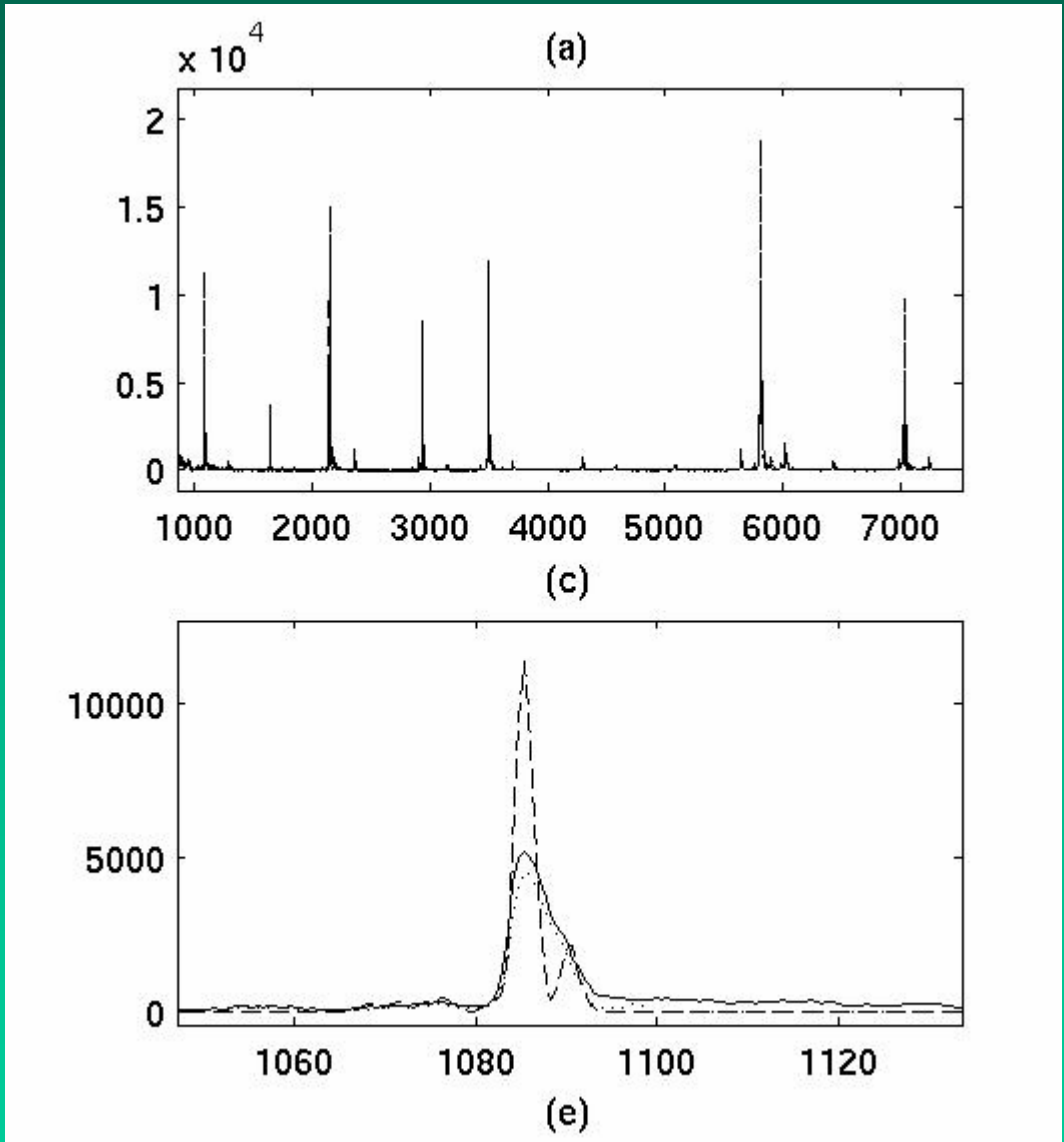


e production depends on velocity, not energy

- FEL could fragment/ionize at detector



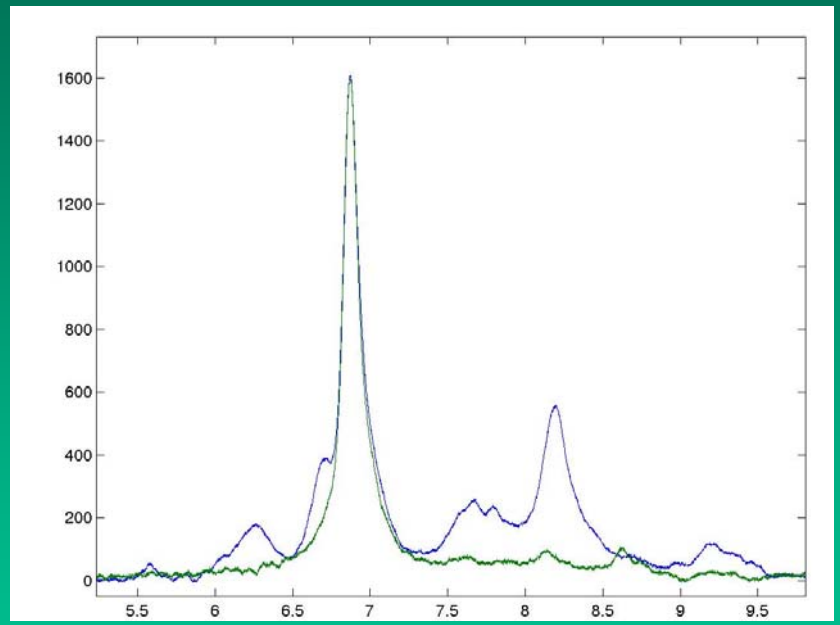
Will the FEL enable higher resolution?



- Filtering resolves hidden peaks
- Can we physically separate them?

Shifted peaks can mean different biology

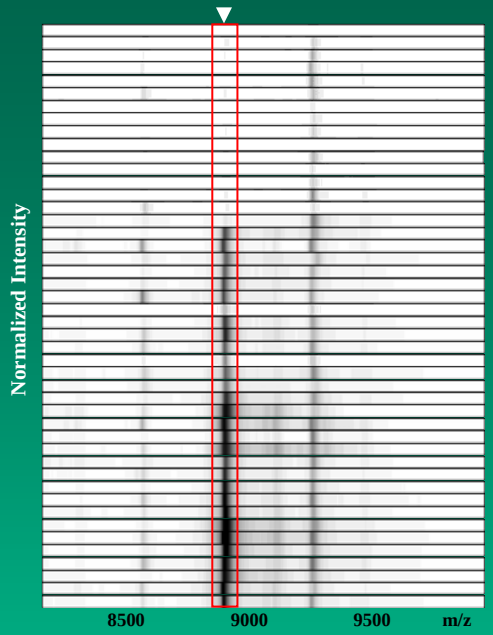
- Proteins come with modifications:
  Phosphorylated
  Acetylated
  Methylated
  Sulfated



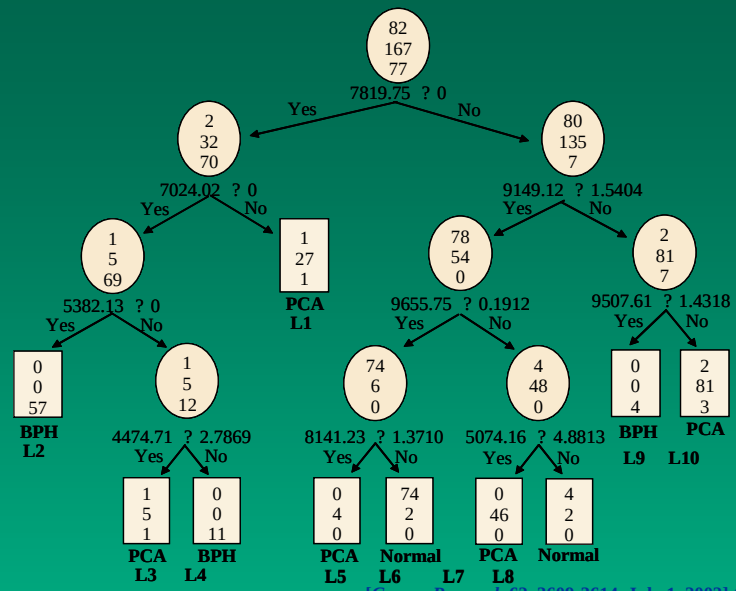
How can you tell if a shoulder is a peak or a problem?

Summary of Biomarker Discovery and Identification

SELDI-TOF

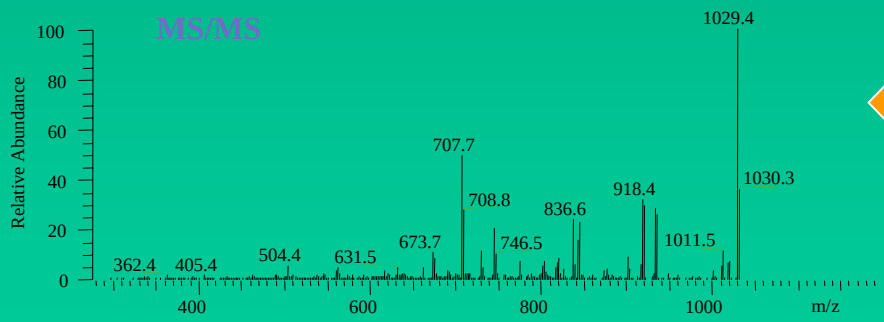


Classification and Regression Tree Analysis

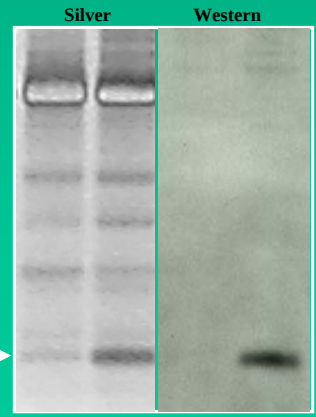


[*Cancer Research* 62, 3609-3614, July 1, 2002]

Identification

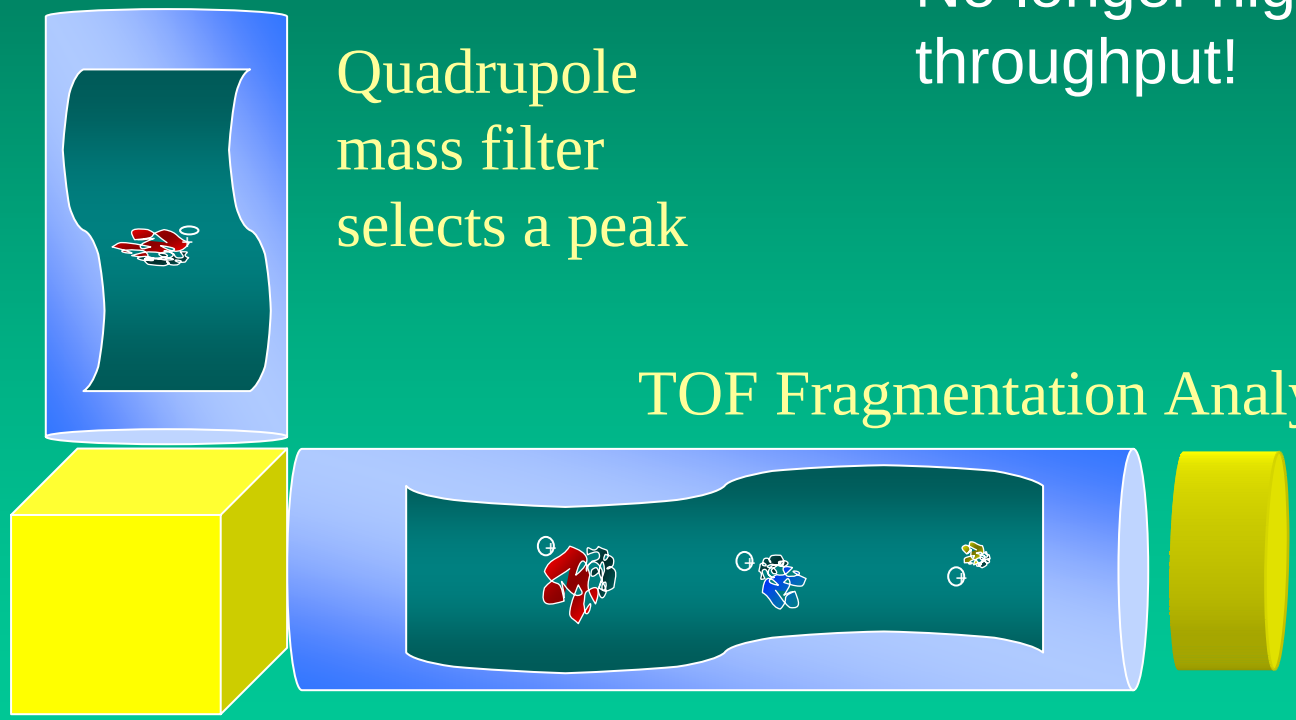


Purification



MS/MS for protein identification

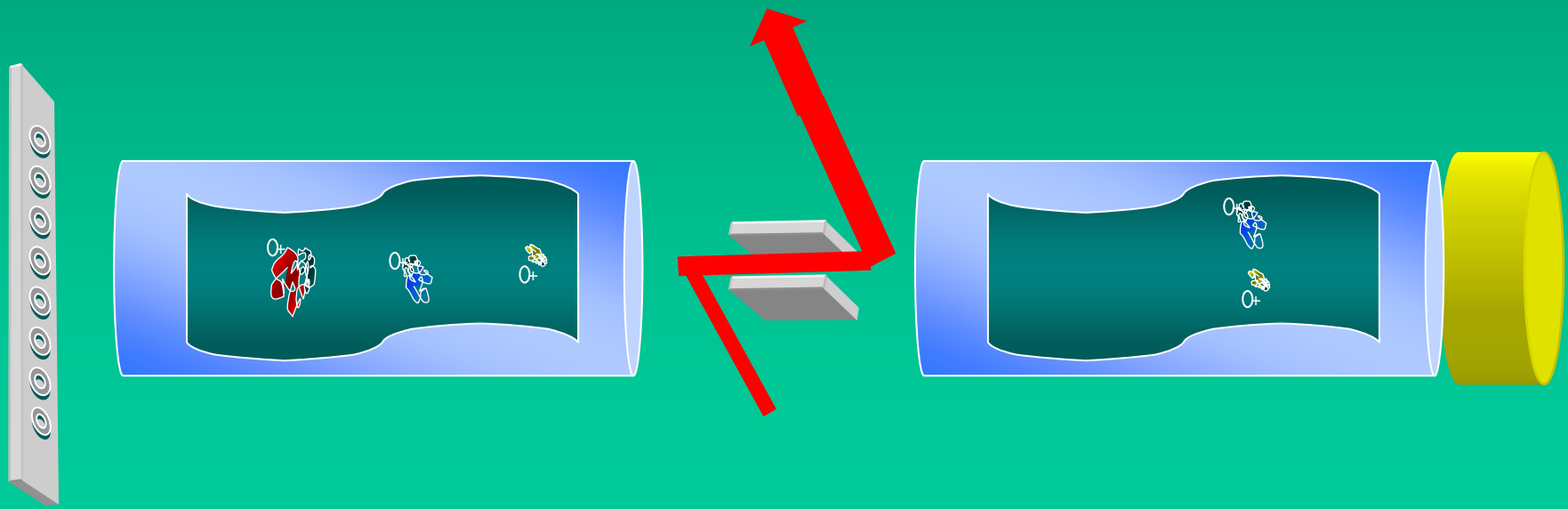
No longer high throughput!



MS/MS for Using FEL Fragmentation/Ionization

With FEL fragmentation/Ionization you can get more than one ion per parent!

- Timing determines the parent mass
- Vertical position determines the fragment mass



The upgraded FEL is the perfect post-ionizer

- High rep rate makes it quasi continuous

$$\Delta x = (30000\ \text{m/s})(12\ \text{ns}) = 360\,\mu\text{m}$$

As long as the Rayleigh range is at least 360 microns, every ion will see a pulse.

The upgraded FEL is the perfect post-ionizer

- High peak power enhances multi-photon ionization or fragmentation

$$I_{\max} = \frac{2\ \text{kW}}{10^{-4}\ \text{cm}^2}\left(\frac{12\ \text{ns}}{0.2\ \text{ps}}\right)\times 100 = 10^{14}\ \text{W/cm}^2$$

The upgraded FEL is the perfect post-ionizer

- Tunability or intensity variation may enhance biological selectivity
- Specifics of fragmentation are unimportant, as long as there is a difference!

Conclusion

- High throughput MS/MS will be possible using the upgraded FEL.
- MS/MS will simultaneously improve resolution and protein identification.
- CW laser post-ionization requires understanding of the ionization and fragmentation processes for biomolecules
- New fast, imaging detectors are necessary